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PRE-APPEAL BRIEF REQUEST FOR REVIEW

		Docket Number: 20751-004001
I hereby certify under 37 CFR §1.8(a) that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to Mail Stop AF, Commissioner for Patents, Box 1450, Alexandria, VA 22313-1450. August 8, 2006 Date of Deposit Lisa G. Gray Signature Lisa G. Gray Typed or Printed Name of Person Signing Certificate	Application Number 09/697,028	Filed October 25, 2000
	First Named Inventor Jeffrey Olson et al.	
	Art Unit 1637	Examiner Suryaprabha Chunduru

Applicant requests review of the final rejection in the above-identified application. No amendments are being filed with this request.

This request is being filed with a Notice of Appeal.

The review is requested for the reason(s) stated on the attached sheet(s).
Note: No more than five (5) pages may be provided.

I am the

☐ applicant/inventor.

☐ assignee of record of the entire interest.
See 37 CFR 3.71. Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96)

☒ attorney or agent of record 35,283
(Reg. No.)

☐ attorney or agent acting under 37 CFR 1.34.
Registration number if acting under 37 CFR 1.34

Signature

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August 8, 2006
Date

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below'.

☐ Total of no. forms are submitted.

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Attorney's Docket No.: 20751-004001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	: Jeffrey Olson et al.	Art Unit	: 1637
Serial No.	: 09/697,028	Examiner	: Suryaprabha Chunduru
Filed	: October 25, 2000	Conf. No.	: 3430
Title	: METHODS FOR GENETIC ANALYSIS OF DNA TO DETECT SEQUENCE VARIANCES		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REASONS FOR PRE-APPEAL BRIEF
REQUEST FOR REVIEW

Applicants respectfully request review of the final rejection mailed February 8, 2006.

Current Claims

The presently pending claims (10-16) relate to DNA amplification methods that cause differential amplification of two nucleic acid molecules that differ in sequence at a polymorphic site. Thus, a first nucleic acid molecule having a first allele at a polymorphic site is amplified to a greater extent than a second nucleic acid molecule having a second allele at the polymorphic site. Such methods are useful, for example, when one wishes to obtain relatively more copes of one of the two nucleic acid molecules for analysis.

The independent claims require the use of two amplification primers, neither of which hybridize to the polymorphic site. One of the two primers includes a "5' portion which, when incorporated into the amplification product, will upon further amplification yield products that form a stable stem-loop structure, the stem of which is perfectly matched and includes the polymorphic site only when the second nucleotide is present at the polymorphic site, but not

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August 8, 2006
Date of Deposit

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Signature

Lisa G. Gray
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when the first nucleotide is present at the polymorphic site". Thus, one of the primers has a 5' region that upon integration into amplification product, will form a stem-loop having a perfectly matched stem containing the polymorphic site when the second allele (second nucleotide) is present at the polymorphic site, but not when the first allele (first nucleotide) is present at the polymorphic site. Since a sufficiently stable stem-loop interferes with further amplification, the formation of a perfectly matched stem in the case of second allele will cause the nucleic acid molecule containg the second allele (the second nucleic acid molecule) to be amplified to a lesser extent than the nucleic acid molecule (the first nucleic acid molecule) containing the first allele. This result is referred to as differential amplification.

Rejection of Claims

The Examiner rejected claims 10-16 under 35 U.S.C. §102(e) as anticipated by U.S. Patent No. 6,326,145 (the '145 patent"). The Examiner, citing Example 1 (col. 12, lines 54-67 and col. 13, lines 1-52) of the '145 patent, argued that the '145 patent discloses as method for achieving differential amplification of two different alleles in a mixture such that a first nucleic acid molecule having a first nucleotide present at a polymorphic site is amplified to a greater extent than a second nucleic acid molecule having a second, different nucleotide present at the polymorphic site. The Examiner stated that the '145 patent disclose differential amplification because the fluorescence signal "is proportional to the amount of the target present in the sample".

Citing other portions of the '145 patent, the Examiner argued that the '145 patent describes a primer that forms a stem loop only when certain sequence are present in a target nucleic acid molecule that serves as template for extension of the primer. Applicants agree that the '145 patent describes a probe that can form a stem loop under certain conditions. However, the '145 patent does not describe a method for achieving differential amplification and does not teach a primer that forms a stem-loop in the manner required by the present claims.

The '145 Patent

The '145 patent describes a detection probe ("Scorpion probe") that is capable of forming a stem-loop when a selected sequence is present in a sample. The Scorpion probe includes a signaling system such a fluorophore and a quencher. The Scorpion probe also includes a template binding region that hybridizes to a template nucleic acid molecule. The Scorpion probe also has a target binding region that can participate in the formation of the stem of a stem-loop structure if the probe is extended to include a sequence complementary to the target binding region. Formation of the stem-loop prevents the flourophore form interacting with the quencher, thereby allowing a fluorescent signal to be emitted when the desired template sequence is present in a sample. As the '145 patent explains, the Scorpion probe is a sensitive means for detecting a particular sequence. The Scorpion probe hybridizes to a sequence within a template nucleic acid molecule via the template binding region. Extension of the Scorpion probe on the target will cause the incorporation into the probe of additional sequence. If extension of the probe creates a sequence that is complementary to the target binding region already present within the probe, then a stem-loop structure forms and a fluorescent signal is emitted. If extension of the probe creates a sequence that is NOT complementary to the target binding region already present within the probe, then the stem-loop structure does NOT form and NO fluorescent signal is emitted. Thus, it can be seen that Scorpion probe is a detection system, not a primer for differential amplification. In fact, the '145 patent explains (col. 5, lines 51-56) that the method described are detection methods that are used in conjunction with amplification methods (col. 5, lines 64-65).

The '145 patent does not teach differential amplification required by the claims

The '145 patent does not disclose a method for achieving differential amplification of two different nucleic acid molecules. The Examiner has failed to demonstrate that Example 1 (or any other portion) of the '145 patent discloses differential amplification. Example 1 discloses a PCR amplification in the presence of a probe ("Scorpion probe") that serves a way to detect a nucleic acid molecule having a particular sequence, e.g., a particular allele. Fig. 13, which

depcits the results of the experiment in Example 1, shows that the fluorescent signal produced by a Scorpion probe that matched the target allele increased with time during a PCR amplification reaction. However, contrary to the Examiner's assertion, this is not an indication that differential amplification has occurred. Indeed there is no indication that two nucleic acid molecules differing by the nucleotide present at a polymorphic site are even present in the experiment, much less that two different nucleic acid molecules were differentially amplified. Thus, nothing cited by Examiner suggests that the '145 patent teaches differential amplification of two molecules that differ in the nucleotide present at a polymorphic site.

Moreover, the '145 patent appears to teach just the opposite of differential amplification. Example 2 of the '145 patent describes the use of two different Scorpion probes -- one that matches the sequence present in the template nucleic acid molecule and one that does not match the sequence present in the template nucleic acid molecule. Each Scorpion probe was added to a PCR amplification reaction containing the template nucleic acid molecule. As shown in Fig. 14, the matched Scorpion probe (i.e., the one having a target binding region perfectly complementary to a sequence in the template nucleic acid molecule) generated a strong fluorescent signal while the mismatched Scorpion probe (i.e., the one having a target binding region that is NOT perfectly complementary to a sequence in the template nucleic acid molecule) did not generate a significant fluorescent signal. The '145 patent explains that "both amplifications were equally efficient" (col. 13, lines 58-60). This in not the result that would be observed if the Scorpion probe was causing differential amplification. If the Scorpion probes were causing differential amplification, the efficiency of the amplification reaction would depend on whether the matched or mismatched Scorpion probe was present in the amplification reaction. Applicants grant that this is a situation with two different probes, not two different templates. However, the experiment shows that the there is the same degree of amplification whether or not a probe is exactly complementary to the target or not.

In view of the forgoing, it is clear that the '145 patent cannot anticipate the present claims.

The probes described in the '145 do not include a 5' portion that is incorporated into an amplification product

One of the two primers used in the presently claimed methods includes "a 5' portion which, when incorporated into an amplification product, will upon further amplification yield products that form a stable stem-loop structure..." The Scorpion probe of the '145 patent forms a stem-loop, but is very different. As the '145 patent explains, when the Scorpion probe is used in an amplification system such as PCR, the Scorpion probe includes a blocking moiety (e.g., hexethylene glycol) that is located between the template binding region and the 5' target biding region that prevents the 5' target binding region from being amplified (see col. 2, lines 54-67). It is this non-amplified 5' target binding region that forms the stem of the stem-loop that forms when the proper target sequence is present. As explained above, it is this stem-loop formation that prevents quenching of the fluorescent signal thus allowing a fluorescent signal to be admitted. Thus, the Scorpion probes do not contain a 5' region that is incorporated into the amplification product and forms a stem-loop structure (when a certain target sequence is present), as required by the present claims. For this second, independent reason, it is clear that the '145 patent cannot anticipate the present claims.

Enclosed is a Petition for Extension of Time with the appropriate fee, a Notice of Appeal with the appropriate fee, and a Pre-Appeal Brief Request for Review. Please apply any other charges or credits to deposit account 06-1050.

Respectfully submitted,

Date: 8 AUG 2002

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